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Tyramide Signal Amplification (TSA)-FISH Applied to Mapping PCR-Labeled Probes Less than 1 kb in Size

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ABSTRACT

Tyramide signal amplification (TSA)-FISH was used to map one mouse and two human DNA probes of less than 1 kb in size. The two human probes were 319 and 608 bp, and the mouse probe was 855 bp. Probes, made from PCR products, were labeled by incorporating biotin-11-dUTP (human) and biotin-16-dUTP (mouse) during PCR amplification. Signals were readily observed in both interphase and metaphase cells following TSA-FISH for all three genes, whereas conventional FISH experiments produced no signals. The two human ATP-binding cassette (ABC) genes, EST883227 (GenBank® Accession No. AA243820) and EST990006 (GenBank Accession No. AA348546), mapped to human chromosomes 7p21 and 17q25. The mouse gene, *c-myc* (exon 2) mapped to band D2 of mouse chromosome 15. These findings demonstrate the ability of this technique to map small probes (PCR products and expressed sequence tags) of less than 1 kb through highly increased signal amplification.

INTRODUCTION

Fluorescence in situ hybridization (FISH) is a technique routinely used for chromosome mapping in humans, mouse and other mammals through visualization of a probe hybridized to metaphase chromosomes and interphase nuclei (2). Conventional FISH involves the labeling of DNA with a reporter molecule (e.g., biotin) by polymerase chain reaction (PCR) or nick translation (16), followed by hybridization of probe and target DNA and incubation with immunofluorescent reagents [e.g., avidin-fluorescein isothiocyanate (FITC)] (16). One limitation of FISH has been the size of the fragment that could be detected once labeled. Conventional FISH has

not proven reliable enough to map single copy probes that are smaller than 1 kb in size.

In this study, we explored the sensitivity of an alternative FISH method, Tyramide Signal Amplification (TSA)-FISH (NEN Life Sciences Products, Boston, MA, USA; <http://www.nenlifesci.com>), which has been used in a number of other applications, such as mRNA in situ hybridization (ISH) on formaldehyde-fixed and paraffin-embedded tissues (15), co-localization of HuC and HuR genes (members of Evi family of RNA-binding proteins) on the same chromosomal preparation (metaphase spreads) using small probes (1.2 and 2.5 kb, respectively) labeled by nick translation (19) and detection of the human cytomegalovirus immediate early (HCMV-IE) gene integration site (17).

TSA-FISH is a multi-step procedure involving in situ hybridization with a labeled probe, detection of a hybridized target with streptavidin-horseradish peroxidase (SA-HRP), signal amplification (incubated with tyramide-biotin), detection of amplified signal [incubation with streptavidin-fluorescein (SA-FITC)] and imaging (10). Amplification is increased greatly in this procedure by the biotin-labeled tyramide, a phenolic compound, that reacts and binds with electron rich moieties (e.g., HRP) that catalyzes the binding of the tyramide to the surface of the cell preparations on the slide. Therefore, tyramide-based detection always requires a peroxidase-conjugated antibody. The key to TSA-FISH is the multiple deposition of the reactive biotinylated-tyramide, resulting in the amplification of a fluorescent signal up to 1000-fold (4,9-11,13,14,18).

The two human probes used in this study EST990006 (GenBank® Accession No. AA348546) and EST883227 (GenBank Accession No. AA243820) belong to the ATP-

binding cassette (ABC) superfamily. These genes are members of a large family of proteins that code primarily for transport proteins involved in the movement of important molecules across cell membranes including lipids, peptides, hydrophobic and chemotherapeutic drugs. ABC genes are involved in a number of human genetic diseases including X-linked adrenoleukodystrophy, cystic fibrosis and Stargardt disease (Reference 1 and <http://www.gene.ucl.ac.uk/users/hester/abc.html>).

C-myc is an proto-oncogene and is a member of the helix-loop-helix/leucine zipper family of transcription factors (6,7). When *c-myc* is activated by mutations, it acts as a cell proliferation regulator in mammalian cells (9). *C-myc* over-expression has been found in many human and murine neoplasias, as for example in plasmacytomas, and was previously localized by FISH in the mouse to chromosome 15, band D2 (3).

As a control, we attempted to map the small, labeled probes by conventional FISH before mapping with TSA-FISH. However, no signals were visible using conventional FISH. Therefore, we proceeded to map the probes by using TSA-FISH with minor modifications in the protocol. This study is the first application of TSA-FISH for mapping of small probes (less than 1 kb in size) labeled by PCR in metaphase chromosome spreads.

MATERIALS AND METHODS

PCR Conditions

The human DNA probes were labeled with biotin-11-dUTP, and the mouse DNA probe was labeled with biotin-16-dUTP using PCR amplification. The human probe was synthesized by PCR from human genomic DNA by the following procedure. For each reaction, the master mixture included 10× buffer containing 15 mM MgCl₂ (PE Biosystems, Foster City, CA, USA), 2 mM dNTP, 5 U/μL *Taq* DNA Polymerase (PE Biosystems), forward and reverse primers (1 μM each) and double-distilled (dd)H₂O up to 24 μL. For the dNTP, the final concentrations were: 0.2 mM each dATP, dCTP and dGTP, 0.15 mM dTTP and 0.03 mM biotin-11-dUTP. The final reaction mixture contained 1 μL of DNA (20–40 ng) and 24 μL of the master mixture. PCRs were incubated at 95°C for 2 min followed by 12 cycles: denature at 94°C for 12 s, anneal at 65°C for 20 s and extend at 72°C for 55 s. The reactions were incubated next for 30 cycles at 94°C for 12 s, 50°C for 20 s and 72°C for 55 s. After completion of the last cycle, the reaction was extended at 72°C for 10 min and held at 4°C. Two repeats of each PCR were pooled, and the OD₂₆₀ read. Primers for EST883227, forward 5'-CCATAGCAGTTATTG-CATTG-3' and reverse 5'-GATCATATAACCTCTGCAG-AAG-3', and EST990006, forward 5'-CGGTTCCATCCAA-CACCTG-3' and reverse 5'-CAATGGAACCAGTATGGC-CTG-3', generated 319- and 608-bp fragments, respectively.

Mouse probe DNA was synthesized by PCR according to the method previously described (8), with the exception that 25% of the dTTP was substituted with biotin-16-dUTP. The following primer pairs, forward primer, 5'-TCCTTC-CTCTCGTTTCCCCGCCCTCTGCTTT-3', and reverse primer, 5'-TGGAAGTGTGTGGAGGTGTATGGGGTGTA-G-3', annealed in the exon 2 region and generated a 855-bp fragment for the *c-myc* locus in mouse.

Fluorescence In Situ Hybridization

Two experiments were run concurrently to measure the sensitivity of TSA-FISH. Mapping of the probes was attempted with both conventional FISH (12) and TSA-FISH. In each experiment, the mapping of probes involved looking for the presence of signals in twenty normal metaphase spreads. For the human probes, metaphase spreads were used from peripheral blood lymphocytes. For both conventional and TSA-FISH, we followed the same protocols up to and including the blocking step, detailed below. Two different amounts of the DNA from the human probes were tested, 0.5 and 3.0 μL (1 μL = 600 ng), by conventional FISH and TSA-FISH.

Slide Pretreatment

Slides were pretreated with 3% hydrogen peroxide (H₂O₂) in methanol (10 min) and rinsed in 1× phosphate-buffered saline (PBS), to minimize background and quench endogenous peroxidase activity. For removal of excess cytoplasm, the slides were then pretreated by using the digestive enzyme, pepsin (Sigma, St. Louis, MO, USA). For pepsin pretreatment, 10 μL of pepsin from a 10% stock solution (100 mg/mL) were dissolved in sterile water placed into 100 mL of pre-warmed (37°C) 0.01 M HCl, pH 2.0–2.5. The slides were placed into a coplin jar containing the pepsin/HCl solution, for 5 min at room temperature. Following pretreatment, slides were fixed with 1% formaldehyde in 1× PBS/MgCl₂ and washed once with 1× PBS (at room temperature). Slides were then dehydrated in an ethanol series, 70%, 90% and 100% for 3 min each (11).

DNA Precipitation, Denaturation and Hybridization

Human probes were prepared by combining 0.5 or 3 μL of probe (DNA concentration 600 ng/μL), 10 μL of Human Cor-1® DNA (500 μg, 1 mg/mL; Life Technologies, Gaithersburg, MD, USA) and 1 μL (10 μg) salmon sperm DNA (10 mg/mL; Sigma). The mouse probe consisted of 25 μL of probe (DNA concentration, 10 ng/μL) and 2 μL salmon sperm DNA.

The probes were precipitated by adding 1/10 volume Na-acetate (3 M) and 2.5–3.0× total volume of cold 100% ethanol, vortex mixed and stored at -20°C overnight or at -80°C for 1 h. Next, the probes were centrifuged at 14 000 rpm at 4°C for 30 min in a Model 5417 R Eppendorf® Microcentrifuge (Brinkmann Instruments, Westbury, NY, USA). The supernatant was removed, and the tubes were placed in a speed-vac for 10 min (at medium heat) to dry the pellet. Then 5 μL of deionized formamide (pH 7.0–7.5) were added to each probe, the tubes were vortex mixed and incubated at 37°C for 30 min (shaking). Five microliters of the master mixture [20% dextran sulfate in 2× standard saline citrate (SSC), pH 7.0] were added to the probe mixture, the tubes were vortex mixed and spun down. The probe DNA was denatured at 85°C for 5 min in a water bath. The slides were denatured by applying 120 μL of 70% formamide in 2× SSC (pH 7.5), covered with a 24 × 60 mm² coverslip and heated at 80°C on a slide warmer for 1.5 min (time may vary depending on slides). The slides were immediately dipped in freshly prepared ice cold 70% ethanol for 3 min, followed by 90% and 100% ethanol dips of 3 min each. The denatured probe DNA was applied to the dry, denatured slides and covered with an

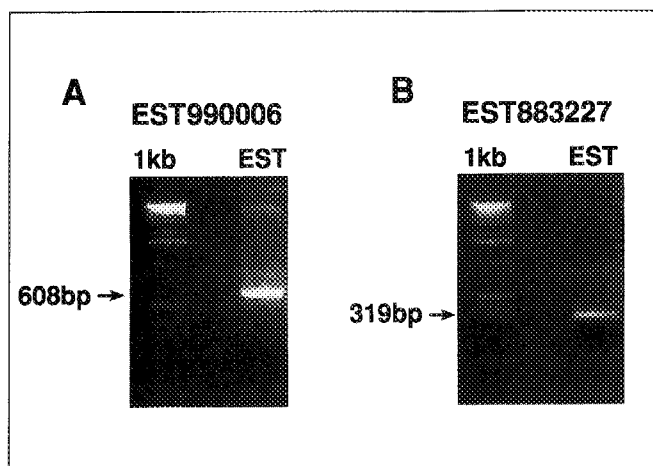


Figure 1. Human probes for EST883227 and EST990006. This figure displays the results of PCR amplification of the two human ABC genes (A and B). The PCR products were run on 2% agarose gels using ethidium bromide staining and a 1-kb ladder as the marker.

18-mm² coverslip, sealed with rubber cement and placed in a light-proof/hybridization chamber for 24–48 h at 37°C.

Detection

Conventional FISH. Slides were: (i) washed three times in 50% formamide/2× SSC (pH 7.0–7.5) (45°C for 5 min each); (ii) washed three times in 0.1× SSC (60°C for 5 min each); (iii) dipped in 4× SSC/Tween® 20 buffer at 45°C; and (iv) incubated in blocking solution [3% bovine serum albumin (BSA) in 4× SSC/Tween 20] for 30 min at 37°C using 120 µL per slide.

Following blocking, the conventional FISH slides were treated with avidin-FITC antibody (dilution 1:200) for 1 h at 37°C, washed 3 times with 4× SSC/Tween 20, stained with 4′6-diamidine-2-phenylindole (DAPI) (for 5 min) and washed in water (for 5 min), covered with antifade reagent (para-phenylenediamine antifade; Sigma).

TSA-FISH. TSA™ Indirect *in situ* Hybridization Kits were obtained from NEN Life Sciences Products (biotinyl

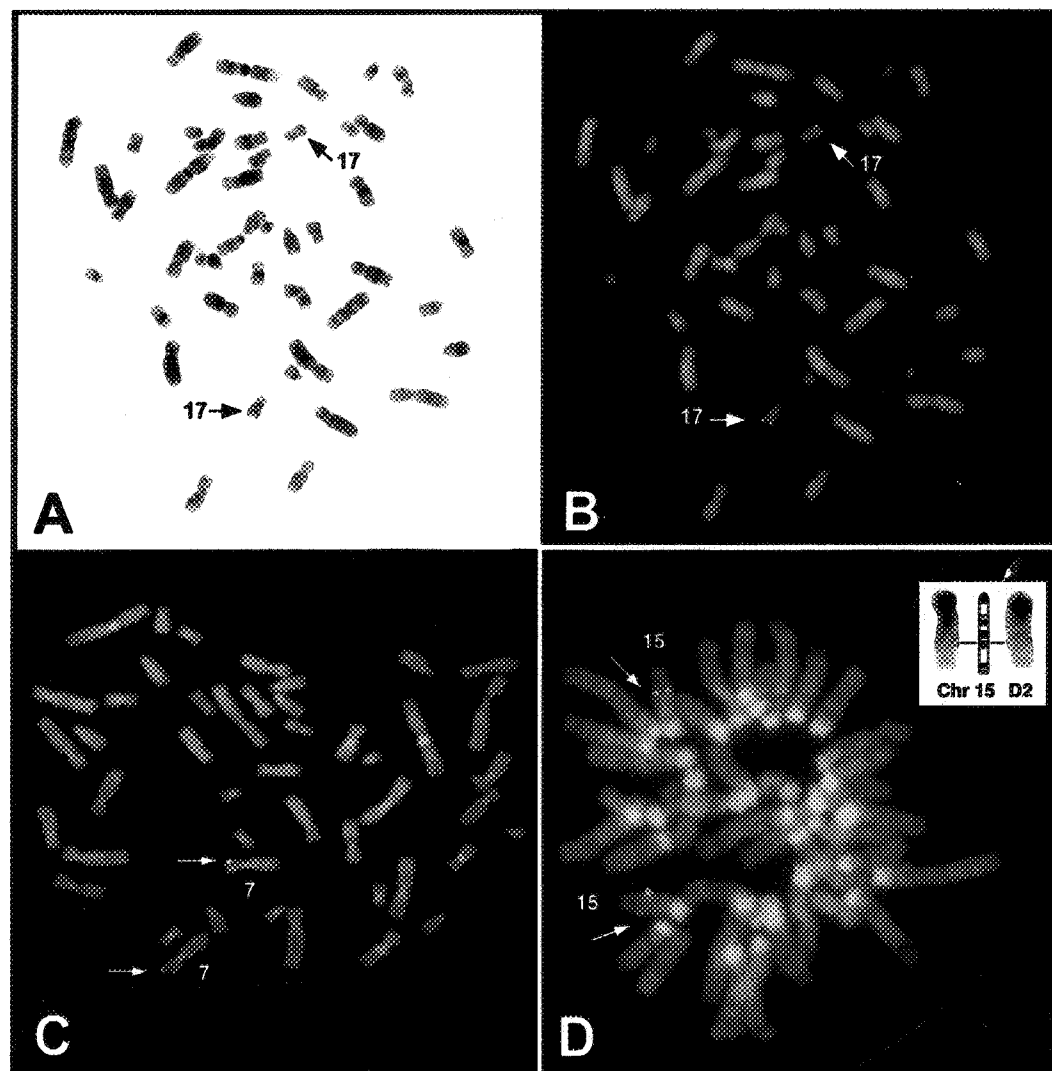


Figure 2. TSA-FISH detection of two human ABC genes and the *c-myc* mouse (exon 2) gene. (A) An inverted-DAPI metaphase spread of a normal human lymphocyte cell. (B) The identical metaphase spread with the chromosomes stained with DAPI (blue). The two green signals at the telomere regions of chromosome 17q25 (arrows) are the result of hybridization with the human ABC gene EST990006 (608 bp). (C) The results of hybridizing the ABC gene EST883227. This probe was 319 bp in size. (D) Mouse metaphase chromosomes with the results of the hybridization of mouse *c-myc*, exon 2 gene (855 bp). The resulting signals, shown in green pseudocolors (arrows), confirm the assignment of this gene to mouse chromosome 15, band D2 (see inset).

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tyramide; Catalog No. NEL 730 for 200–600 slides and NEL 730A for 50–150 slides). TSA-FISH detection followed the protocols as described by the manufacturer with minor modifications. Higher stringency washes ($0.1\times$ SSC) were used to reduce background, and the TNT buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween 20) was brought to pH 7.0–7.5.

The biotin label was detected with SA-HRP (supplied in the kit) 1:100 in TNB [0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Blocking Reagent (supplied in kit)] (120 μ L per slide) for 30 min at room temperature and washed 3 times for 5 min each in TNT buffer (room temperature), while shaking.

For the tyramide amplification procedure, three different methods of detection were tested: (i) direct detection using tyramide-Cy3 (Tyr-Cy3); (ii) direct detection using tyramide-fluorescein (Tyr-fluorescein); and (iii) indirect detection using biotin-avidin-FITC (tyramide bound to biotin; Tyr-Bio).

To each slide, 300 μ L of each tyramide conjugate were applied at various dilutions (Tyr-Bio 1:50; Tyr-Cy3 1:500; Tyr-fluorescein 1:250) without use of a coverslip, for 5 min at room temperature. The tyramide solution was removed, and the slides were washed 3 times for 5 min each with TNT at room temperature. Fluorochrome-conjugated avidin (Av-FITC) diluted 1:200 in TNB was used to detect Tyr-Bio. Slides were incubated for 30–45 min at 37°C. Slides were then washed with TNT buffer 3 times for 5 min each at room temperature, counterstained with DAPI (80 ng/mL in $2\times$ SSC) for 5 min at room temperature, and rinsed in water for 5 min, while shaking. The slides were dehydrated sequentially in 70%, 90% and 100% ethanol. To each slide, the antifade was applied and covered with a coverslip (24 \times 60 mm²) before image acquisition.

Image Acquisition

Fluorescent signals were imaged separately with the appropriate filter set using a Zeiss Axioskop® (Carl Zeiss, Thornwood, NY, USA) or Leica Epifluorescence Microscope (Leica Imaging Systems, Cambridge, England, UK) equipped with a cooled Model CH250 charge-coupled device (CCD) camera (Photometrics, Tucson, AZ, USA). The filters used with a Leica DMRBE Microscope (Leica Imaging Systems) were TR1 (DAPI), TR2 (FITC) and TR3 [tetramethylrhodamine isothiocyanate (TRITC)] (Chroma Technology, Brattleboro, VT, USA). The DAPI and FITC images were merged and pseudocolored using Image™ (Oncor, Gaithersburg, MD, USA) or Q-FISH® (Leica Imaging Systems) software as described previously (11).

All of our images were initially imaged with the Q-FISH software, which reduces background automatically when images are merged, but does not enhance the images otherwise when they are captured (Figure 3). In addition, we find with TSA-FISH, the signals are brighter than those visualized with conventional FISH, and therefore require reduced exposure times so as to not saturate the images of the signals.

RESULTS

We explored the limits of sensitivity of TSA-FISH for gene mapping of probes of less than 1 kb in size. Twenty metaphase spreads from normal human lymphocyte and/or mouse spleen preparations were analyzed for TSA-FISH and routine FISH.

TSA-FISH resulted in bright, amplified signals, whereas conventional FISH methods gave no detectable signals from the human and mouse probes. The indirect method (Tyr-Bio) using avidin-FITC provided the best results, i.e., clear, distinct signals on one or both of the homologues; whereas both the Tyr-Cy3 and Tyr-fluorescein resulted in high background. For EST990006, we observed two signals (doublets) on both homologues of chromosome 17, in the long arm, on all twenty metaphases. For EST883227, the 319-bp probe, we obtained the following data from the 20 metaphase spreads examined: four cells had no signals; four had one signal (on one homologue of chromosome 7 in the short arm); eight had one signal on both homologues of 7p (Figure 2C); four had 3 signals (2 on one homologue and one on the other homologue of 7p); and 0 metaphase spreads were seen with 4 signals or symmetrical doublets on both homologues of chromosome 7.

Using TSA-FISH, we successfully mapped EST990006, EST883227 and the *c-myc* gene using small PCR indirect labeled (biotin) DNA probes [608 and 319 bp (Figure 1) and 855 bp, respectively]. EST990006 mapped to human 17q25, EST883227 mapped to human 7p21 and *c-myc*, as expected, mapped to band D2 on mouse chromosome 15 (Figure 2). The cDNA of these two human ABC genes had been previously sequenced and determined to be unique through Basic Local Alignment Search Tool (BLAST) searches of the expressed sequence tag (EST) and nonredundant databases (<http://www.ncbi.nlm.nih.gov/BLAST/>). TSA-FISH mapping was undertaken after repeated attempts to map the first gene, EST990006, using the GeneBridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, AL, USA). The second gene, EST883227 was previously mapped using the GeneBridge 4 Radiation Hybrid Panel to 7p15-p21.

Human EST990006 is a contig containing the human est H65920 (Genbank Accession No.) and is in the unigene cluster Hs.38095. Cluster Hs.38095 has been mapped by the Sanger Centre (Cambridge, England, UK) to human chromosome 17 (D17S794-D17S795; <http://www.ncbi.nlm.nih.gov/>).

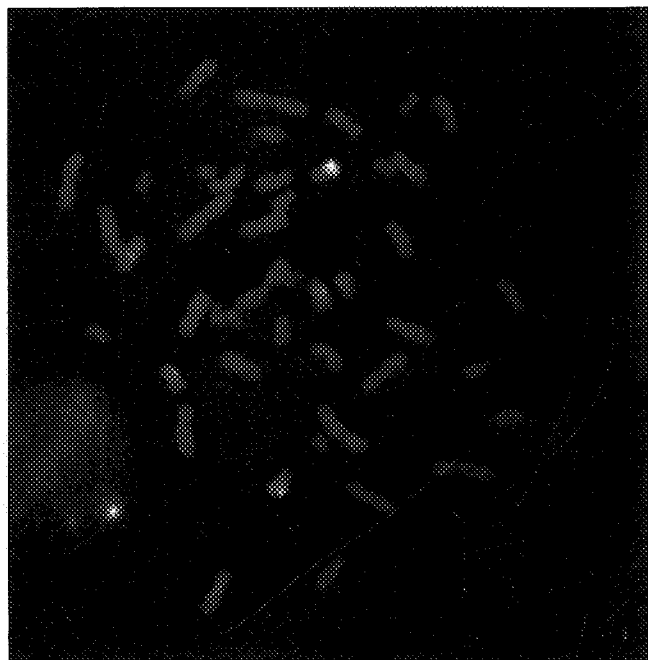


Figure 3. Raw picture of an EST990006 spread. Image before background reduction.

gov/genemap98/). This mapping result is consistent with the TSA-FISH mapping.

DISCUSSION

TSA-FISH is a relatively new protocol that allowed us to detect and to map probes as small as 855, 608 and 319 bp via increased signal amplification. However, as is often observed when mapping very small probes with conventional FISH, the smaller the probe, the lower the frequency of signals (symmetrical doublets) detected in metaphase spreads (5). With the 319-bp probe (Figure 2C) we were able to confirm the physical mapping of the gene, however the probe produced a low frequency of paired doublets, thus reflecting the limits of sensitivity of TSA-FISH for this gene sequence. However, with TSA-FISH, we found a much higher efficiency than expected, with 80% of the EST883227 metaphase spreads having at least one signal on each homologue.

Inherent with using small probes, many more spreads must be examined to have confidence in your mapping results. In addition, the technique is more sensitive to problems associated with high background. Therefore, with TSA-FISH, higher concentrations of probe are not recommended, as they would create greater background. These results have demonstrated the ability of TSA-FISH for mapping cDNAs using PCR-labeled probes. We also mapped for the first time, the human gene EST990006, a new human ABC gene, to 17q25.

The demonstrated sensitivity of TSA-FISH suggests this method might be applicable for the detection of other small sequence specific probes such as PCR fragments, microsatellites and sequence tag sites (STSs). As is true for conventional FISH mapping methods, several components are critical to insure success of the TSA-FISH protocol. The concentration of the DNA and the size of the probe should be determined before hybridization by running the PCR product on a gel and reading the OD₂₆₀. Regarding the concentration of the DNA, an average of 500 ng gives a good signal upon hybridization. Slides with metaphase spreads used for TSA-FISH must be clean and free of cytoplasm to ensure strong hybridization, decreased background and to raise the signal to noise ratio. The time of pepsin treatment and the denaturation time will vary for each preparation of metaphase spreads and must be optimized to ensure proper hybridization.

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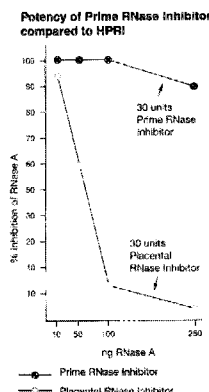
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Nikon's Super Fluor objectives (S Fluor series) for Eclipse upright and inverted research microscopes deliver, according to the company, the highest signal-to-noise ratio available, offering peak transmission over an extended range from the ultraviolet (UV) through the infrared (IR).

The S Fluor series' performance is on a par with Nikon's Apochromats. Nikon CFI₆₀ objectives have high chromatic correction and also offer high NAs and long working distances, even at the highest magnifications. Special coatings and cement assure reduced autofluorescence.

S Fluor optics are an excellent choice for UV excitations of 340 nm or where there is a need for higher transmission to obtain a brighter fluorescence image. Relevant applications include multiphoton, uncaging, laser tweezers, ablation, FRET, FRAP, FLIP and FISH. A 40× phase contrast objective is among the optics offered.

Nikon

Circle No. 267

High-Performance, Flexible Stereomicroscope

The recently introduced high-performance Leica MZ9.5 stereomicroscope has a standard magnification range from 6.7 to 80× and 9.5:1 zoom for biological and life science applications.

Computer-controlled optical design systems were used to develop lenses using new types of glass with enhanced optical properties. The result is a stereomicroscope that offers excellent resolution, image contrast and sharpness. The flexible, modular design allows users to add accessories and customize the stereomicroscope for specific applications. All components and accessories retrofit to existing Leica products, making it easy to adapt the microscope to future applications. According to the company, they offer the widest range of accessories to meet all requirements in the lab.

Leica

Circle No. 270

Biotinylation Reagent Selection Guide

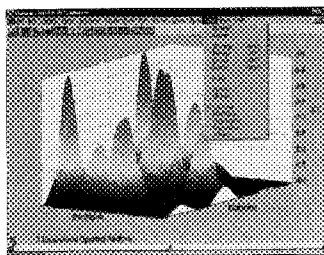
A wall chart makes selection of the correct biotinylation reagents for your experimental applications an easier task. This 2' × 4' chart covers antibody production purification, modification and assay development. Labeling of antibodies, peptides or oligonucleotides with biotin allows the creation of a number of assay formats. Choosing the most appropriate Pierce EZ-Link™ Biotinylation Reagent is usually a four-step process of (i) determining which functional group is available on your molecule, (ii) selecting an available reactive group on the reagent, (iii) identifying the type of linkage formed, and (iv) selecting an EZ-Link that contains this active chemistry.

Pierce

Circle No. 271

Fluorescence Software Features 3-D Spectral Display

Windows® 95/98 and NT-compatible HyperRF software is now available for their RF-5301PC Spectrofluorophotometer. This highly versatile, full featured software package enables multi-component sample analysis and provides three-dimensional spectral plots. HyperRF provides powerful quantitative analysis tools to facilitate a wide range of life science and environmental applications. The new HyperRF software provides full NT security and complete GLP/GMP compliance functions including password protection and report trails. Highly flexible data management capability includes data sharing, import and export, and enables customized report layouts.



Shimadzu

Circle No. 272

Environmental Chamber Between Reach-In and Walk-In Types

The Insta-Room is an environmental chamber designed to fill the price and size gap between refrigerator-sized reach-in units and larger, more expensive, walk-in structures.

Reach-in units offer a maximum space of 75 cubic feet. Larger 10' x 10' walk-ins are about 2 1/2 times the cost of Insta-Rooms, which offer 110 cu. ft. of space. Delivery time is about three weeks, which is substantially shorter than many manufacturers.

The 4" thick panel's R-30 insulation value helps keep temperatures consistent throughout the box. To permit installation in tight locations, the panels holding the room together can easily be joined or separated using W.A. Brown's cam lock system. The Insta-Room offers temperature control accurate to $\pm 0.5^{\circ}\text{C}$. Three types are available: $2^{\circ}\text{--}40^{\circ}\text{C}$, $0^{\circ}\text{--}30^{\circ}\text{C}$ and $4^{\circ}\text{--}40^{\circ}\text{C}$.

Carolina Equipment Consultants

Circle No. 273

Anti-Human HTRF Conjugate

A generic, off-the-shelf, anti-human IgG mouse monoclonal antibody reagent, conjugated with either Europium Cryptate (Eu)K, the primary fluorophore, or XL665, the secondary fluorophore of HTRF assay technology, was raised against the Fc fragment of human IgG. Therefore, it can also be used to verify accessibility of human IgG Fc fusion proteins by competitive binding. For example, assays can be developed using a new anti-human antibody and streptavidin/biotin to complete the FRET (fluorescence resonance energy transfer) pair. Use these new anti-human generic reagents to rapidly develop, mix and measure homogeneous, time-resolved fluorescence assays for pennies per well.

Packard's HTRF reagents can be measured most efficiently on the Discovery HTRF Microplate analyzer, available in two models, both capable of measuring both 96-well and 384-well microplates. The Discovery HTS is the highest throughput model, which includes robotic interface, bar code reader and 40 plate stackers. The Discovery AD system is specifically designed for assay development of HTRF assays with the same high quality optics, but reduced plate capacity and fewer automation features that are not required in assay development labs.

Packard

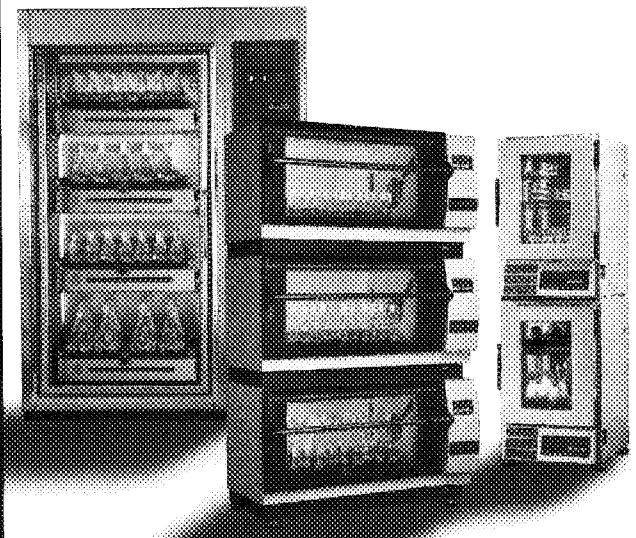
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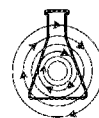
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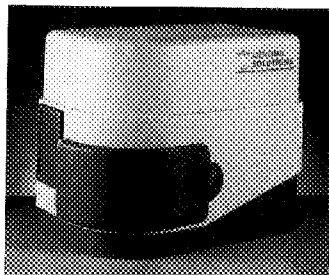
www.nbsc.com

Circle Reader Service No. 243

New Products for Bioresearch

High-Throughput, Fully Automated Biochip Imager

Genomic Solutions announces the launch of the GeneTAC™ 2000 BioChip Analyzer, part of the company's GeneTAC™ Biochip Solution. The GeneTAC 2000 utilizes a powerful detector to capture images with a true 16-bit dynamic range. The sample carousel automates imaging and analysis of up to 24 fluorescently labeled biochips at one time.



The images captured by a high-resolution detector are true 16-bit dynamic range. Quantum efficiency of the detector is 60%–70% vs. 15% for standard PMTs. This quantum efficiency leads to greater sensitivity and faster image capture. The excitation wavelength is determined by an ingenious filter system that allows for the detection of up to four fluorescent labels. Through the use of an automated sample carousel, the GeneTAC 2000 can image up to 24 slides at a time.

Genomic Solutions

Circle No. 275

Expression Analysis Kits with Molecular Beacons for Real-Time PCR

Sentinel™ molecular beacon expression analysis kits are highly specific for cDNA sequences due to the design of the molecular beacon and the primer pair, and can ensure highly accurate quantitative results. These molecular beacon systems are specific for cDNA targets and virtually eliminate interference from possible DNA contamination or pseudogenes. Stratagene offers a variety of expression analysis kits that include control/housekeeping targets and targets useful for investigating inducible gene expression (including mouse β -actin, human GAPDH, human TNF- α , human β -2 microglobulin and human α P2).

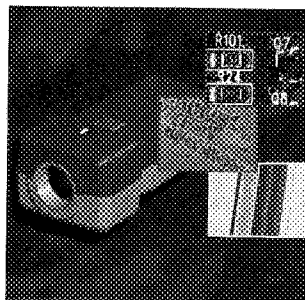
Stratagene

Circle No. 276

Digital Documentation System for Industrial Applications

The Kodak Digital Science MDS 100 allows you to capture high-quality still pictures and smooth video at up to 30 frames per second on or off your microscope. With the USB connection, the MDS 100 delivers live preview, extremely fast, uncompressed images and there's no frame grabber board to install.

With the MDS 100, create video clips and time lapse movies for training/work instructions or high-resolution still images of product defects for documentation and future reference. Applications for the MDS 100 can be found in quality assurance, research, manufacturing and inspection laboratories. The MDS 100 makes digital imaging easy to use and even easier to afford. The included software allows you to digitally zoom in for detail and easily capture, edit and enhance images. Drivers for Windows 98 and Windows 95 version B are included.



Kodak Scientific Imaging Systems

Circle No. 277

Genomic DNA Isolation

DNAzol® is a complete and ready-to-use reagent for the isolation of genomic DNA from solid and liquid samples of animal and plant origin. The reagent contains a novel guanidine-detergent lysing mixture that promotes selective precipitation of DNA in a fast and reliable protocol. Genomic DNA isolation can be completed in 30 min with DNA recovery of 70%–100%. Fifty milliliters of DNAzol is adequate to process 50 samples of 25–50 mg of tissue.

Molecular Research Center

Circle No. 278

RNase Decontamination Solution

ElectroZap™ safely eliminates RNase and DNase contamination from glass and plastic surfaces. This noncorrosive solution is especially designed for the decontamination of pH meter electrodes. It is essential that electrodes are RNase-free for the preparation of solutions which cannot be DEPC-treated such as Tris-HCl. ElectroZap is simply sprayed onto the electrode and then rinsed off with RNase-free water. ElectroZap works instantaneously, leaving no residue that may interfere with subsequent enzymatic reactions.

Ambion

Circle No. 279

Chemiluminescence Analysis System

ChemiGenius, a system that uses Peltier-cooled 16-bit CCD cameras for dark current noise elimination and sophisticated software for gel analysis and documentation, has been announced. According to the company, the system captures the ultra-faint emissions of chemiluminescent indicators in a fraction of the time previously required for film exposure while recording a much wider dynamic range of output. The chemiluminescence technique eliminates the use of radioactive indicators and their accompanying health and safety concerns. Once acquired, the images can then be analyzed using GeneTools Match software, which performs automatic band matching, molecular weight analysis, volumetric measurement, reference track selection, reference track libraries and extensive results formats—all at the click of a button.

The ChemiGenius is available with two camera resolutions: the standard model has a resolution of 694 × 494 pixels, while the ChemiGenius Plus has a resolution of 1300 × 1030 pixels.

Syngene

Circle No. 280

Two Efficient Polycationic Liposome Formulations for DNA Transfection

Two polycationic liposome formulations complement Quantum's successful viral gene delivery technology. GeneSHUTTLE™ reagents are non-classical, novel liposome reagents made with proprietary, non-glycerol backbone-based polycationic lipids. According to the company, the unique chemical functionality present in these lipids imparts them with superior transfection properties and sets them apart from other reagents. For instance, GeneSHUTTLE-20™ has the ability to transfect, with high selectivity, primary cells; whereas GeneSHUTTLE-40™ transfects DNA and RNA into a variety of cultured as well as primary cells in the presence of 10% serum. In addition, GeneSHUTTLE-40 is, according to the company, an outstanding reagent for the transfection of insect cells such as Sf9.

Quantum Biotechnologies

Circle No. 281